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Original Research

Beta-catenin or Pax 2. Which one is more useful in endometrial cancer?

Biomarkers in endometrial carcinomas

Devrim Kahraman
Department of Pathology, TOBB Economics and Technology University School Of Medicine, Ankara, Turkey

Abstract

Aim : Pax 2 is a nuclear transcription factor. It is essential for the embryonic development of Müllerian organs and is suppressed through at later stages of embryonic development, but is reactivated during carcinogenesis. Beta- catenin is a protein that is translocated from membrane to cytoplasm and nucleus in WNT activation as a signaling pathway. Endometrioid carcinoma is associated with beta- catenin mutations. This study aimed to evaluate PAX2 and Beta-catenin expressions in benign and precancerous endometrial hyperplasias.

Material and Methods: The study was performed on 40 endometrial curettage materials, including benign endometrial hyperplasia (n: 20), precancerous endometrial hyperplasia (n: 10), and endometrioid carcinoma (n: 20) as study groups. For immunohistochemical evaluation, one representative paraffin block for each case was selected.

Results: Pax 2 nuclear staining was detected in all endometrial tissues. The mean percentage was % 70 in benign hyperplasia and % 90 in precancerous endometrial hyperplasia and endometrioid carcinoma. Beta- catenin membranous-cytoplasmic staining was detected in only precancerous endometrial hyperplasia with a percentage of % 80 and endometrioid carcinoma with a percentage of % 90.

Discussion: Pax 2 is expressed in benign endometrial hyperplastic, precancerous endometrial hyperplasia and carcinoma, but beta catenin is expressed in only precancerous endometrial hyperplasia and carcinoma. These findings suggest that both the WNT signaling pathway and PAX 2 transcription factor may contribute to the development of endometrial cancer.

Keywords

Cancer Precursor; Endometrial Hyperplasia; Endometrioid Carcinoma; Cancer Biomarkers

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Corresponding Author: Devrim Kahraman, TOBB economics and technology university school of medicine, Department of Pathology, TOBB Hospital Ankara Yasam caddesi no:5 pk:06510 Söğutozu/Ankara, Turkey.
E-mail: dr.devrimsonmez@gmail.com P: +90 5055250295
Corresponding Author ORCID ID: https://orcid.org/0000-0001-9858-9252

Introduction

Endometrial cancer is the most common gynecological malignancy, which is responsible for approximately %4 of all cancers in women worldwide [1]. Endometrial hyperplasia is an irregular proliferation of endometrial glands with an increased gland stromal ratio and a benign process . When it is combined with atypia, the WHO classification system categorises it as "atypical endometrial hyperplasia [EH]" or "endometrial intraepithelial neoplasia [EIN]" (ESGO – European Society of Gynaecological Oncology. Pocket Guidlines _ Endometrial Cancer; 2017). The categorization is based on histomorphological criteria [2,3], and histological examination is the standard between benign and premalignant EH. It may cause additional problems, especially in poor inter- and intra-observer evaluations.

PAX 2 is a member of the paired box gene family. There are nine members from PAX1 to PAX9. PAX 2 is expressed during embryonic development and organogenesis [4]. Also, PAX proteins play a critical role in various types of malignancy [5,6]. In the literature, the immunohistochemical evaluation of PAX2 has been recommended to distinguish premalignant lesions in endometrial hyperplasia [7]; but this can also be seen in benign endometrial hyperplasia. Therefore, we need support for a definitive differential diagnosis.

Beta-catenin plays a role in WNT activation as a signaling pathway. Aberrant activation of the WNT/Beta- catenin pathway has been reported in patients with endometrial cancer [8]. Beta- catenin can be a good sporter for distinguishing precancerous lesions and focal malign areas.

In this study, we aimed to evaluate PAX2 and Beta- catenin expressions in precancerous endometrial tissues and malignant focal areas in hyperplastic endometrial fragments.

Material and Methods

Case selection:

In this study, we used paraffin blocks of curettage materials of 60 endometrial tissues. We evaluated them in three diagnostic categories as follows: 20 benign endometrial hyperplasias, 20 precancerous endometrial hyperplasias, and 20 endometrial carcinomas. The samples were particularly chosen from the population of reproductive age and postmenopausal period. Histological classification of benign and precancerous endometrial hyperplasia was based on the World Health Organization criteria (ESGO – European Society of Gynaecological Oncology. Pocket Guidlines _ Endometrial Cancer; 2017). The tumors were staged according to the 2009 Federation Internationale de Gynecologie et d'Obstetrique (FIGO) staging system [9]. The histological grading was also based on the FIGO system.

Immunohistochemical analysis:

With the examination on hemotoxylin and eosin (H&E) stained slides of the samples, one representative block for each case was selected for immunohistochemical evaluation. Sections with 3 µm thickness were cut from the blocks and incubated with PAX2 rabbit anti-human polyclonal [pSer393] antibody [isotype: IgG, catalog ID/Lot ID: LS-B2450/27017, Lifespan Biosciences, Seattle, USA] at a dilution of 1:100 for 35 min, in an automatic immunostainer (BenchMark XT Staining Module, Ventana Medical Systems Inc; Tucson, AZ, USA) using the streptavidin Biotin complex immunodetection system. Antigen retrieval was achieved with CC2 (citrate, pH:6) solutions (Ventana) and protein blockage was applied. Diaminobenzidine was used for chromogen, followed by hematoxylin counterstaining. Fetal kidney tissue was used as a positive control. In the examination, only the specific nuclear staining was considered positive. In all cases, nuclear staining was detected in various (of moderate or strong intensity) percentages. The percentage of positive nuclei was achieved by counting 1000 epithelial endometrial cells. An alternative scoring system was also applied with reference to Monte et al [10].

Results

All endometrial tissues showed moderate to strong staining in endometrial epithelial cells with PAX2. All precancerous endometrial hyperplasia and endometrial carcinoma samples showed moderate to strong staining with Beta-catenin. Immunoreactivity was only nuclear in PAX2 and membranous-cytoplasmic in Beta- catenin. Non-specific staining was not observed in both biomarkers.

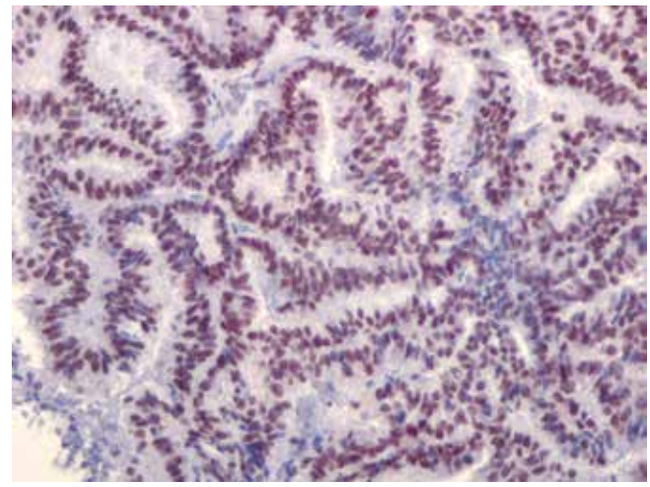

Figure 1. PAX2 in malignant endometrial curettage. [PAX2X200]

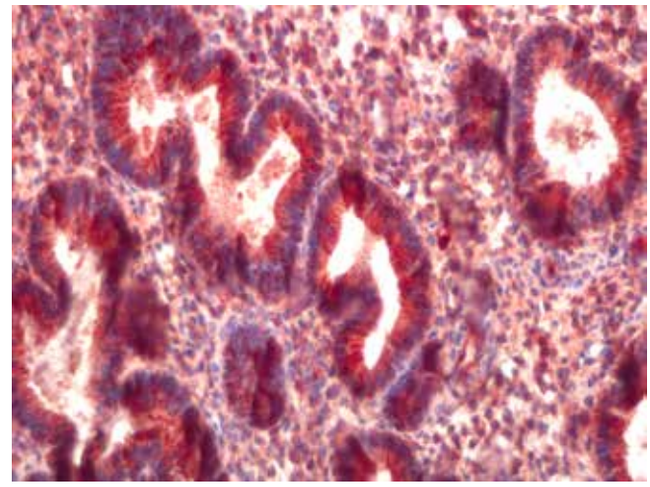

Figure 2. Betacatenin in precancerous endometrail curettage. [BetacateninX200]

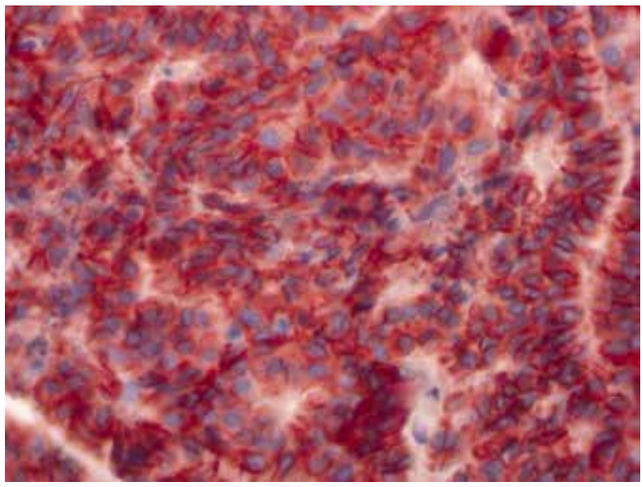

Figure 3. Betacatenin in malignant endometrial curettage. [BetacateninX200]

PAX2 expressions varied in quantity in benign endometrial hyperplasia, it was diffuse and strong in precancerous endometrial hyperplasia and endometrial carcinoma (Figure2).
Beta-catenin expressions were not seen in benign endometrial hyperplasia, it was diffuse and strong in precancerous endometrial hyperplasia (Figure 2) and endometrial carcinoma (Figure 3).
Differences among subgroups were marked. The positivity rate with PAX2 was increased in the sequence of benign endometrial hyperplasia, precancerous endometrial hyperplasia, and endometrioid carcinoma. Whereas beta-catenin positivity was seen only in precancerous endometrial hyperplasia in endometrioid carcinoma.

Discussion

The present study revealed the role of Beta-catenin expressions in precancerous endometrial hyperplasia and endometrial carcinoma. When used with PAX2, the degree of neoplasia can be demonstrated with more supportive findings. We found that all of the endometrial samples (benign endometrial hyperplasia, precancerous endometrial hyperplasia and endometrioid carcinoma) showed moderate to strong nuclear immunoreactivity to PAX2. Staining reactivity was strongest in endometrial carcinoma. It was lowest in benign endometrial hyperplasia. These findings are in accordance with the other studies showing that PAX2 plays a role in endometrial carcinogenesis [11-16]. Staining with Betacatenin provided a significant benefit in the differential diagnosis of carcinoma from benign tissues. Whereas beta-catenin positivity is seen only in precancerous endometrial hyperplasia and in endometrioid carcinoma, the role of beta-catenin in colon carcinogenesis has been known for a long time [14]. However, the effect of beta-cetanin on endometrial cancer has only just been revealed. It is reportedly implicated in endometrial and ovarian carcinogenesis [17]. Dysfunction in the activation of the WNT/Beta-catenin pathway can promote cancer development [18]. Jun-Jiang et all. reported abnormal WNT/Betacatenin signaling in endometrial cancer. They found a significant correlation between multi-drug resistance protein 4 with betacatenin mRNA levels in endometrial cancers, in particular at stages I and IV. Also, Coopes A et al. showed aberrant activation of WNT/Beta-catenin in endometrial cancer [8]. The level of beta-catenin inside the cell is a key to the canonical Wnt/Betacatenin signaling [14]. In 2009, Jeong JW et al. found that beta-catenin mediates glandular formation. They also showed that dysregulation of beta-catenin induces hyperplasia formation in the murine uterus [15]. Several prior studies found different results about the relationship between Beta-catenin mutations and recurrence and its effect on prognosis. Myers A et al. found nine times higher odds of Betacatenin mutation in tumors of those patients who recurred compared to those who did not [16]. In 2012, Byron et al. evaluated disease-free survival in stage I or II endometrial cancer and could not find any difference based on Beta-catenin status [17]. In 2017, Katherine C. et al considered all stages of endometrial carcinomas, and as a result, they found that Beta-catenin mutant tumors were associated with higher rates of grade 1-2 disease. They found low rates of Beta-catenin in deep myometrial invasion and in lymphatic/vascular space invasion [18]. Thus many previously published studies have indicated the importance of beta-catenin in carcinogenesis [19-24]. In this study, we studied the comparison of beta-catenin and PAX2 in endometrial carcinogenesis for the first time with benign, precancerous and malign tissues. Thus, we can say that Beta-catenin mutation is an indicator of cancer, but the effect on prognosis is still controversial in the literature.

Scientific Responsibility Statement
The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement
All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest
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